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Isolation,cloning and molecular analysis of *ag85a* and *tb10.4* genes from *Mycobacterium tuberculosis*

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Background and Objective: Novel TB vaccines that aim to boost and/or replace Bacillus Calmette-Guerin (BCG) are currently in development. DNA vaccines can stimulate both humoral and cell-mediated immunity in different animal models of TB and is thought to be a promising strategy in the development of new vaccines against TB. The aim of this study was to design and construct a DNA vaccine encoding *ag85a* and *tb10.4* fusion genes of *Mycobacterium tuberculosis*

Materials and Methods: *tb10.4* fragment was amplified by PCR and the products were digested with restriction enzymes. Next, it was cloned into the pcDNA3.1$^+$ plasmid. The *ag85a* gene and pcDNA3.1$^+$/*tb10.4* plasmid were digested by *Eco*RI and *Bam*HI restriction enzymes. Constructed vector was sequenced and molecular analysis was done by using bioiinformatic software such as DNAMAN. New chimeric vector containing Ag85a-tb10.4 genes were purified and for confirming expression of pcDNA3.1$^+$/*tb10.4-ag85a* plasmid in eukaryotic cells ,Huh7.5 cell lines were transfected with this recombinant vector.

Results: Using electrophoresis of PCR products, fragments 297 bp for *tb10.4* and 1017 bp for *ag85a* were observed.alignment of ag85a-tb10.4 genom sequence with reference genes in GenBank showed exact identity that indicate correction of aii cloning procedures. Eukaryotic cells transfection with pcDNA3.1$^+$/*tb10.4-ag85a* vector was confirmed with cDNA synthesis and existence of *tb10.4-ag85a* fusion gene was confirmed with RT-PCR(Fig1,2,3,4).

Conclusion:

In this study, *tb10.4 and ag85a* genes were isolated from *Mycobacterium tuberculosis* H37Rv strain and cloned into pcDNA3.1$^+$ also in this study capability of constructed vector in producing fusion Ag85a-tb10.4 protein was confirmed with RT-PCR. pcDNA3.1$^+$/*tb10.4-ag85a* vector can be used for further studies in future.

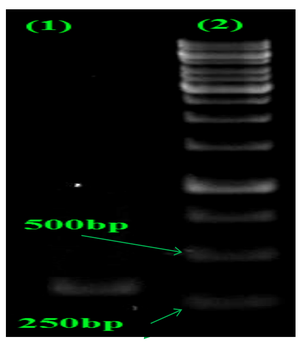

Fig.1: Purified PCR product of *tb10.4* gene on 1.5% gel : Lane 1: 290 bp band of *tb10.4* , Lane 2: 1kb DNA size marker (SM0313, Fermentas, Germany)

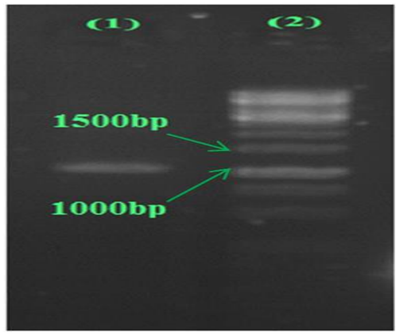

Figure 2: Purified PCR product of *ag85a* gene on 1.5% gel : Lane 1: 1017 bp band of *ag85a* , Lane 2: 1kb DNA size marker (SM0313, Fermentas, Germany)

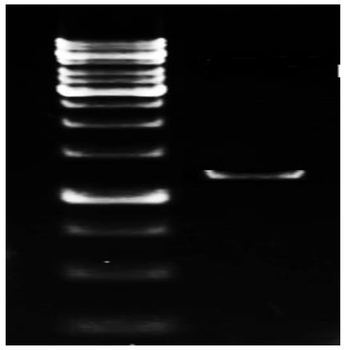

Fig 3: Restriction enzyme digestion of pcDNA3.1$^+$/*ag85a-tb10.4.* Lane 1: 1kb DNA size marker (SM0313, Fermentas, Germany). Lane 2: 1300 bp band of *tb10.4*/*ag85a*.

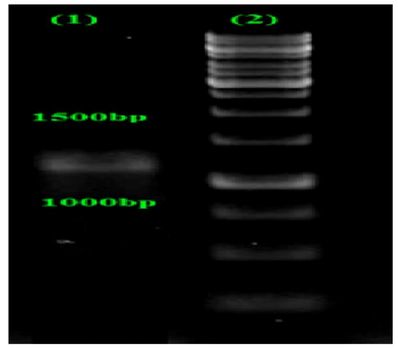

Fig4: RT-PCR results. Lane 1: 1300 bp band of *ag85a*/*tb10.4*.Lane 2: 1kb DNA size marker (SM0313, Fermentas, Germany).

References

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